Applied StemCell, Inc.
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Datasheet

TARGATT™ Human Induced Pluripotent Stem Cell (hiPSC) Research Grade (GMP Matching) Master Cell Line & Knockin Kit

Product Information

Catalog Number AST-9450

Description

The TARGATT™ hiPSC Research Grade (GMP Matching) Master Cell Line and transgenic kit were designed for fast and site-specific knockin in human iPSC cells, using an easy-to-use gene knockin approach. The master cell line provided in this kit contains an “attP” integrase-recognition landing pad engineered into the hH11 safe harbor locus in the genome. The kit also contains a cloning plasmid containing a corresponding “attB” sequence into which any gene of interest can be cloned (under control of the CAG promoter). The expression of the integrase (provided as an integrase plasmid) mediates the stable integration of the transgene into the master cell line (ASE-9450-C). The TARGATT™ integrase technology enables highly efficient site-specific integration after antibiotic enrichment, without disruption of internal genes. The TARGATT™ iPSC master cell line and knockin kit are ideal for single transgene knockin and uniform, stable expression of your protein*.

The TARGATT™ iPSC Master Cell Line has been engineered from our well-characterized, karyotype normal, control iPSC line, ASE-9280. The ASE-9280 parental iPSC line was reprogrammed using episomal factors from CD34+ Umbilical Cord Blood Cells with full consent from male donor. Both the parental ASE-9280 line and the AST-9450-C TARGATT™ iPSC Master Cell Line have been characterized for pluripotency biomarkers, normal karyotype, and directed-differentiation to three germ layers as a validation of functional pluripotency.

The TARGATT™ iPSC master cell line and knockin kit are suitable for research applications involving site-specific large transgene knockin, gene overexpression, and other stable cell line generation applications*.

**We also have TARGATT™ master cell lines in HEK293 and CHO backgrounds. TARGATT™ master cell lines can also be generated in any cell line. Please contact Applied StemCell for TARGATT™ cell line engineering services to generate a master cell line in a specific cell line-of-choice.*

Advantages of using TARGATT™ Master Cell Lines for gene knockin:

- Site-specific and high efficiency integration
- Stable knockin cell line generation
- Single copy gene integration into safe harbor locus
- Gene expression from an active, intergenic locus
- Low off-target integration

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Parental Line	Control human iPSC (ASE-9280) Gender: Male Tissue Source: CD34+ Umbilical Cord Blood Cells Reprogramming Method: Episomal
Clinical information	Healthy (with no known disease phenotypes)
Contents	All cell lines and plasmids provided in this kit are sufficient for 3 transfections according to the given protocol: • TARGATT™ hiPSC Research Grade (GMP Matching) Master Cell Line; AST-9450C • TARGATT™ CAG-Integrase Plasmid; AST-3084 • TARGATT™ 46 CAG-MCS Cloning Plasmid; AST-3091
Quality Control	A certificate of analysis (COA) with detailed quality control information for the cell line and components of the kit will be provided with each shipment.
Shipping	Dry ice
Storage and Stability	Store the TARGATT™ master cell line in liquid nitrogen freezer immediately upon receipt. Store the plasmids at -20°C. Do not freeze-thaw plasmids repeatedly. The products provided in this kit are stable for at least 6 months from the date of receiving when stored as directed.
Safety Precaution	**PLEASE READ BEFORE HANDLING ANY FROZEN VIALS**. Please wear the appropriate Personal Protective Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.
Restricted Use	This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Schematic Representation of TARGATT™ Knockin Strategy

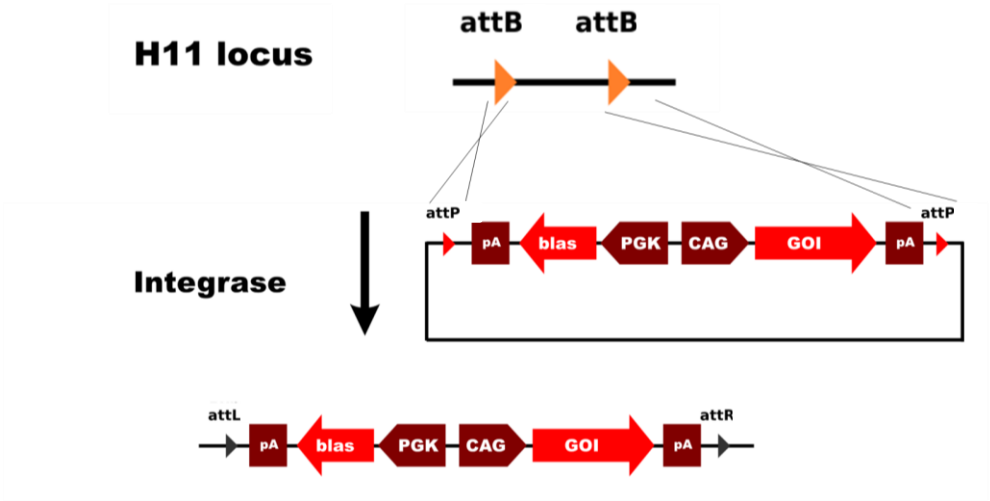

Figure 1. Schematic representation of TARGATT™ site-specific transgene integration mediated by integrase.

Media and Material

Catalog #	Component	Amount
ASE-9450-C	TARGATT™ hiPSC Master Cell Line	1x10^6 cells/vial
AST-3091	TARGATT™ 46 CAG-MCS Cloning Plasmid	5 µg
AST-3084	TARGATT™ CMV-integrase Plasmid	15 µg

Materials Needed but not Provided

Material	Vendor	Catalog#
mTeSR™ Plus	Stem Cell Technologies	05825
Matrigel® hESC-Qualified Matrix, LDEV-free	Corning	354277
CloneR	Stemcell technologies	05888
CryoStor® CS10 Freeze Media	Stemcell Technologies	210102
0.5mM EDTA in PBS	Life Technologies	15575-020
TrypLE™ Express Enzyme (1X), No Phenol Red	Gibco	12604013
Lonza™ Transfection System (100 µL) Kit	Lonza	V4XP-3024
Hygromycin	InvivoGen	Antihg-1
Penicillin-Streptomycin (100X)	Gibco	15140-122
Ganciclovir (GCV)	Selleck Chemicals	S1878
Blasticidin	Invivogen	ant-bl-05

The above reagents are recommended based on our culture protocols. If you are using a similar/ alternative reagent, we recommend that you perform a small-batch test using your preferred reagents.

Protocol

1. Thawing and culturing cryopreserved cells

To ensure the highest level of viability, thaw the vial containing the cells and culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1.1 Prepare 4 mL of mTeSR™ Plus media (containing 1X Pen/Strep) with 10 µM Rock Inhibitor (mTeSR™ Plus media + Rock Inhibitor).
1.2 Add 1 mL of mTeSR™ Plus media + Rock Inhibitor in one well of a Matrigel® coated 6-well plate. Prepare 2 wells for each vial of frozen cells.
1.3 Use dry ice to bring one vial of frozen ASE-9211-TGT-PH3 cells to the cell culture room. Quickly thaw it in a 37°C water bath by gently shaking the cryovial continuously until only a small piece of ice remains.
1.4 Wipe the cryovial with a paper towel sprayed with 70% ethanol and place in a biosafety hood.
1.5 Add 9 mL of mTeSR™ Plus in a 15 mL-conical tube.
1.6 Use a 5 mL serological pipette to transfer the cells to the 15 mL tube.
1.7 Centrifuge the cells at 200 x g for 5 minutes at 4°C.
1.8 Aspirate the medium and add 2 mL of mTeSR™ Plus media + Rock Inhibitor.
1.9 Gently flick the conical tube to resuspend the cells and transfer them to the 2 wells of the Matrigel® coated plate.
1.10 Place the plate in the incubator and move the plate back-and-forth and side-to-side twice to spread the clumps evenly in the wells.

2. Sub-culturing procedure (culture maintenance)

2.1 The next day, aspirate the medium and add 2 mL of fresh mTeSR™ Plus into each well.
2.2 Change the medium every day. For medium change, aspirate off the spent medium and add 2 mL of fresh mTeSR-plus medium in each well.
2.3 When the colonies are big enough or close to merge, the cells need splitting.
Note: To ensure the best quality of cells, the cell culture should be passaged every 4-6 days.
2.4 Aspirate the medium from the culture dish.
2.5 Wash once with 1 mL of 0.5 mM EDTA (in PBS).
2.6 Aspirate the PBS, add 1 mL of 0.5 mM EDTA per well and incubate the cells for 3 minutes in a 37°C incubator.
2.7 Observe the cells under the microscope. The cells at the edge of the colonies will start to separate and round up.
2.8 With the cells still attached, aspirate the EDTA and add 1 mL of mTeSR™ Plus medium.
2.9 Scrape the cells from the bottom of the well until all the colonies are floating; pipette up and down 2-3 times to break the colonies into small clumps.
2.10 Make a 1:10 dilution and transfer the cells to the wells of the new Matrigel® coated plates.
2.11 Place the plate in the CO_2 incubator and move the plate back-and-forth and side-to-side twice to spread the clumps evenly in the wells.

3. Cryopreserving cells

3.1 Label the cryovials as needed, based on 2 vials per well of a 6-well plate, and pre-chill them in a 4°C freezer.
3.2 Aspirate the medium from the hiPSC culture.
3.3 Wash once with 1 mL of 0.5 mM EDTA (in PBS).
3.4 Aspirate the PBS and add 1 mL of 0.5 mM EDTA per well in 6-well dish and incubate the cells for 3 minutes in a 37°C incubator.
3.5 Observe the cells under microscope until the cells at the edge of the colonies start to separate and round up.
3.6 With the cells still attached, aspirate the EDTA and add 2 mL of CryoStor® CS10 medium.
3.7 Scrape the cells from the bottom of the well until the colonies are all floating.
3.8 Aliquot the cell suspension in 2 pre-chilled and labeled cryovials: 1 ml in each vial.
3.9 Place the cryovial in a CoolCell® Freezing Container or in a Styrofoam rack at - 80°C overnight, and transfer to liquid nitrogen the next day.

4. Transfection procedure

4.1 Plasmid amplification and cloning with TARGATT™ 46 CAG-MCS cloning plasmid

4.1.1 Amplify the plasmids using NEB® 10-beta Competent E. coli (High Efficiency) competent cells
Note: Do not use 5-alpha competent cells to transform the plasmids.
4.1.2 Clone the gene of interest into the TARGATT™ 46 CAG-MCS Cloning Plasmid to make the donor plasmid.
4.1.3 Aliquot into single-use quantity and store at -20°C. Do not freeze-thaw the plasmid repeatedly.
Note: We recommend using 5 µg of the plasmid for each transfection

4.2 Transfection using Lonza® 4D-nucleofector transfection system

4.2.1 Culture the TARGATT™ hiPSC cells until the cells reach a 50% confluency.
4.2.2 Replace the complete medium with warm medium without Pen/Strep medium and culture overnight.
4.2.3 Aspirate the medium.
4.2.4 Wash the cells once with 1 mL PBS.
4.2.5 Add 1 mL TrypLE into each well and incubate for 5 minutes at 37°C.
4.2.6 Mix the cells by pipetting about 5 times and transfer the cells into a 15 mL conical tube.
4.2.7 Add 5 mL of mTeSR™ Plus medium to neutralize the enzyme.
4.2.8 Centrifuge at 200 x g for 5 minutes at 4°C.
4.2.9 Aspirate the supernatant and resuspend the cells in 1 mL PBS and count cell density.
4.2.10 Transfer 1 million cells into a 1.5 mL Eppendorf tube® and centrifuge at 200 x g for 5 minutes at 4°C.
4.2.11 Aspirate the supernatant and resuspend the cells in 100 µL transfection mixture (Table 1) containing the gene of interest cloned into the TARGATT™ 46 plasmid (donor plasmid) and 1 µg of integrase plasmid (AST-3084) and Lonza buffer.

Mixture Table 1. Preparation of the transfection mixture

Reagent	Amount
Donor plasmid containing transgene	4 µg
TARGATT™ CMV-integrase plasmid (AST-3084)	1 µg
P3 Primary Cell Nucleofector™ Solution	82 µL
Supplement 1	18 µL
Final volume	**100µL**

4.2.12 Load the cell-DNA mixture into Nucleocuvette™ Vessel and electroporate the cells using the Lonza™ electroporation system and the following conditions: CA-137.
Note: If using other transfection methods, please optimize your protocol accordingly.
4.2.13 After electroporation, transfer the cells into 3 wells of a Matrigel® coated 6-well plate, culture in non-Pen/Strep mTeSR™ Plus medium + Clone R for 24 hours.
4.2.14 After 24 hours, the medium is refreshed with complete mTeSR™ Plus medium.
4.2.15 The cells will recover in 3-4 days.

4.3 Enrichment protocol

Selection strategy using Blasticidin:
The Blasticidin treatment (positive selection) can remove the non-transfected cells as well as enrich for cells with transgene integrated into the H11 locus.

4.2.1 Three (3) days after electroporation, Expand the cells 20-fold. Use Tryple- Select to digest the cells as single cells.

4.2.2 Plate the single cells in new plate and after 24-hours of culture, replace the growth medium with Blasticidin selection media. (growth media + Blasticidin (10 ug/mL)).

4.2.3 Maintain the selection pressure for 2 weeks, or until the desired level of selection is achieved (can be monitored by checking for mCherry fluorescence using microscope.

4.2.4 Expand the cells for further downstream processing.

4.4 Single cell cloning

4.4.1 On day 12 post-electroporation, dissociate the cells using 1 mL Trypsin LE for 5 minutes into single cells (following step 4.2.5).

4.4.2 Seed the single cell iPSCs into a Matrigel® coated 96-well plate with 80 µL of mTeSR™ Plus medium + Rock Inhibitor, and sort the cells using BD-Melody FACS machine.
Note 1: If you are using a FACS machine by a different manufacturer, please optimize your sorting protocol.
Note 2: If you do not have a FACS instrument or do not want to use FACS for single cell sorting, you could also do a serial dilution.

4.4.3 When the single cell clones grow into 4-8 cell clusters in about 4-5 days, replace the spent medium with 100 µL fresh mTeSR™ Plus medium.

4.4.4 Change the medium every 2 days. For medium change, aspirate off the spent medium and add 100 µL of fresh mTeSR-plus medium in each well.

4.4.5 When the cells reach 10% confluency, transfer each well of cells from the 96-well plate into one-well of a fresh Matrigel® coated 48-well plate.

4.4.6 When the cells reach 60% confluency, passage 50% of the cells into fresh Matrigel® coated 6-well plates and use the other 50% of the cells for genotyping.

Supporting Data

Characterization of the TARGATT™ hiPSC Master Cell Line, ASE-9450-C

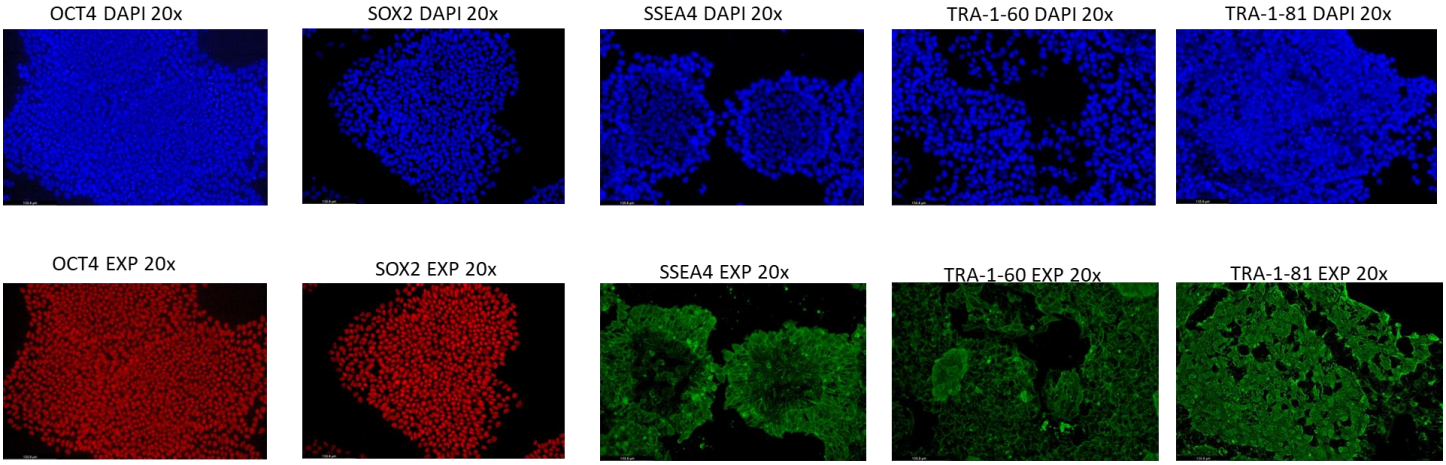

Figure 2. Expression of pluripotency markers. The TARGATT™ hiPSC Master Cell Line, ASE-9450-C expresses common iPSC biomarkers (bottom row: OCT-4, SOX2, SSEA4, TRA-1-60, TRA-1-60, and TRA-1-81). Top row: Corresponding DAPI nuclear staining. All images were taken at 20x magnification.

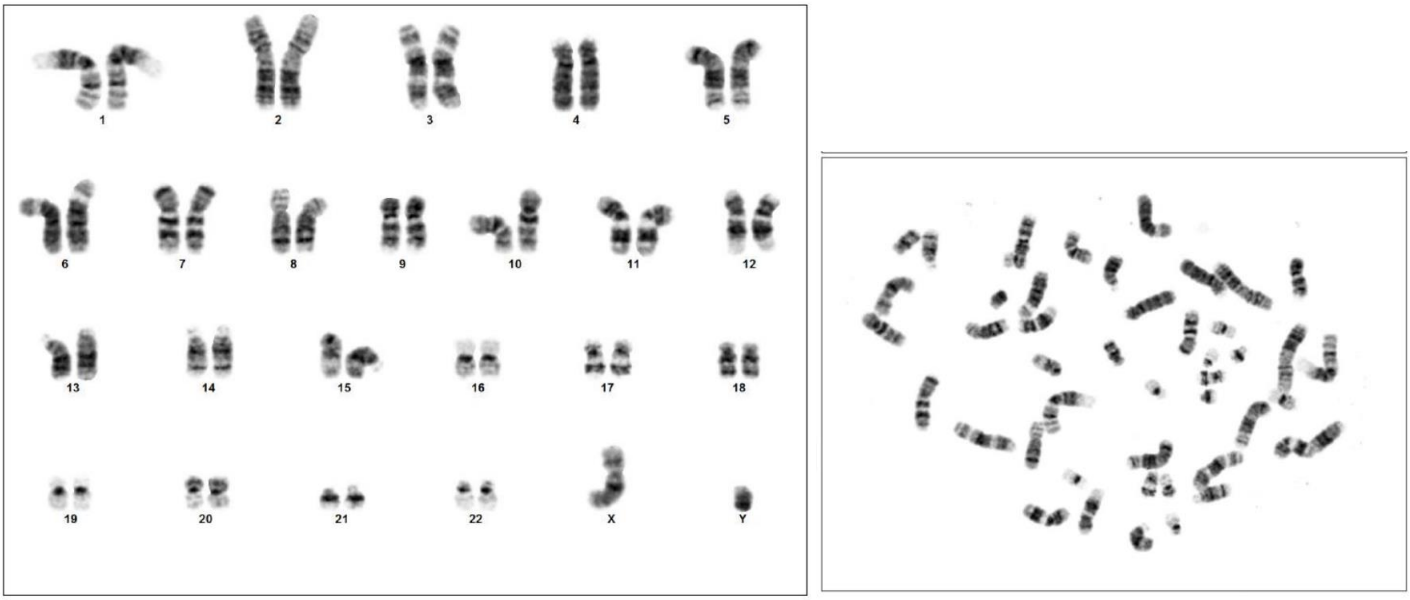

Figure 3. Karyotype (G-banding) and Short Tandem Repeat (STR) Analysis. The cytogenetic analysis performed on the twenty G-banded metaphase spreads of the human cell line ASE-9450-C displayed an apparently normal male karyotype.

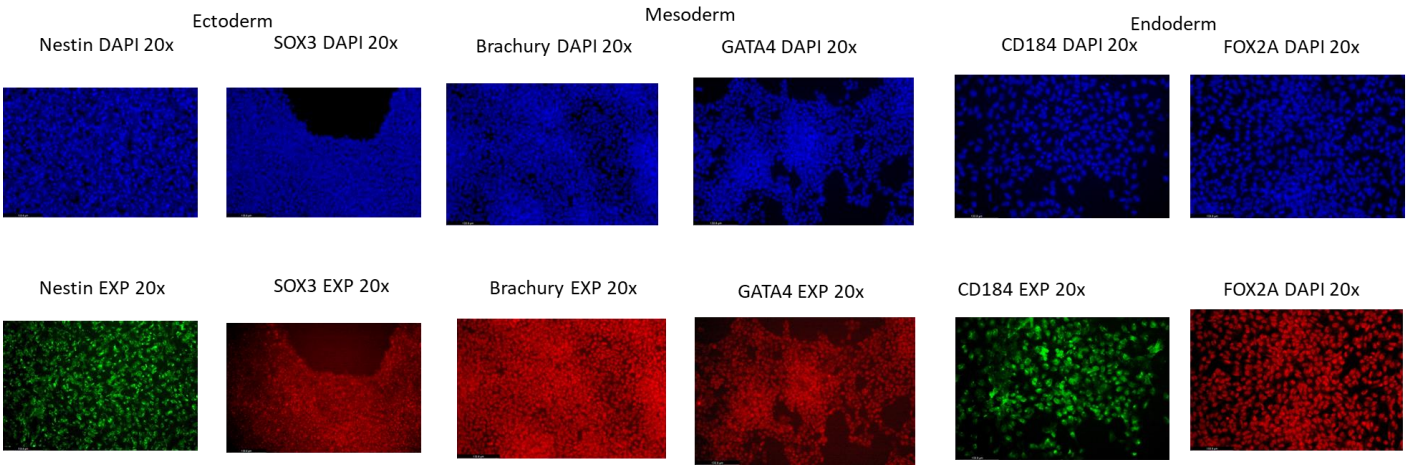

Figure 4. Directed differentiation of TARGATT™ hiPSC Master Cell Line, ASE-9450-C to the three germ layers.
The ASE-9450-C line was differentiated to specific lineages of the germ layers using well-established and optimized protocols. Immunostaining for biomarkers of each lineage was performed to confirm lineage commitment. Cells were also co-stained with nuclear marker, DAPI (blue). Bottom row: Ectoderm (EC) markers: Nestin and SOX3; Mesoderm (ME) markers: Brachury and GATA4; Endoderm (EN) markers: CD184 and FOX2A. Top row: Corresponding DAPI nuclear staining. All images were taken at 20x magnification.

Confirmation of Stable Transgene Expression After Locus-specific Integration into TARGATT™ hiPSC Master Cell Line

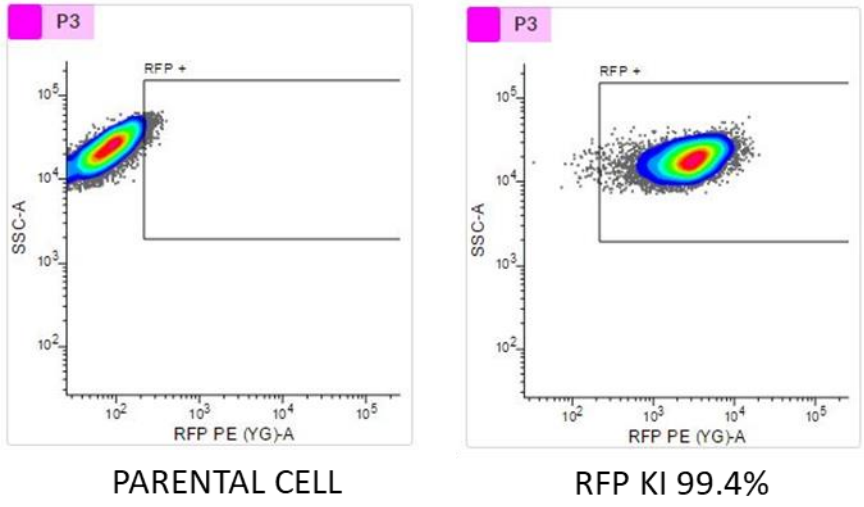

Figure 5. Red fluorescent protein (RFP) integration into the TARGATT™ iPSC Research Grade (GMP Matching) Master Cell Line. The integration was mediated by a unique integrase. The cells were transfected with an RFP-containing plasmid and the provided TARGATT™ integrase plasmid. The integration efficiency of RFP knockin into landing pad was 99.4%, after drug selection.

Appendix

Primer Set for Knockin of Gene of Interest (GOI) Confirmation

The following primers can be used for genotyping analysis to confirm integration of the AST-3091 plasmid:

- **5’ arm H11 primer AGCATGGGAAATAAGTATCACCT**
- **3’ arm H11 primer CCACACTTGTAGTCCCAGCT**

These primers can be paired with each one of your 5’ and 3’ primers derived from your GOI.